Integration of Electron Scan and Light Imagery in Study of Molluscan Radulae

BY

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(1 Plate; 4 Text figures)

INTRODUCTION

OUR PERCEPTION OF THE NATURAL WORLD is strongly molded both by the nature of our sensory apparatus and the characteristics of the investigative tools we employ, to an extent that is seldom explicitly discussed in science. Too often we assume that intellectual objectivity is the only cornerstone of good science. In particular, we place a great deal of faith in our instrumentation.

Optical microscopy (OM), typically utilizing absorption contrast imagery, has long been the standard method of studying the molluscan radula, and most illustrations in the literature consist of camera lucida drawings, composite drawings, or reconstructions based on optical examination. Advances in photomicrography make it possible to produce relatively good photographs containing information not conveyed in artistic renditions, although few workers have used this tool in publication. The advantages of scanning electron microscopy (SEM) have been amply demonstrated in the literature, and SOLEM (1972) has predicted that these advantages "will combine to make optical examination of radular structure obsolete."

Having experimented with all of the above methods of observation and communication, it is clear to me that, while SEM is the most powerful tool in radular study, (1) different tools of observation (i. e., different imaging processes) show us substantially different things, and (2) no single graphic method of communication can transmit all that our instruments show us.

This paper demonstrates the manner in which data from light and electron scan images can be integrated to provide maximum information about radular form and function. First, I review the uses, advantages, and disadvantages of each method. Second, I present specific examples of alternative views of radulae from the phasianellid *Tricolia pullus* (Linnaeus, 1758) and the trochid *Solariella nuda* Dall, 1896.

Effective basic techniques for mounting radulae for OM and SEM examination are readily available in the literature (see especially MEEUSE, 1950; SOLEM, 1972) and are not reviewed herein. Black-and-white photomicrographs in this paper were taken with an American Optical Series 10 Microstar trinocular microscope with Spencer Model 600 Ortho-Illuminator, AO Photostar Exposure Meter and 35 mm camera back. Scanning electron micrographs were taken with the Cambridge Mark II-A and S4-10 instruments at the U. S. National Museum of Natural History with a Tectronix oscilloscope camera Model C-27.

The following discussion and illustrations are based on morphological and evolutionary studies of radulae of rhipidoglossate marine archaeogastropods (HICKMAN, 1976, and in preparation). Optical microscopy and scanning electron microscopy are combined to document convergent radular configurations that include: (1) complex within- and between-row basal overlap and interlock systems; (2) within-row shaft interlock and cusp interlock systems; (3) a variety of modifications to simultaneously accomplish close packing of teeth when the radula is collapsed and maximum reach when it is in use; (4) disproportional development of the outermost lateral tooth cusp, in extreme cases with compensatory development of radular asymmetry; (5) systems of pivotal or articulatory plates in the hinge regions between the portions of the radula functioning along different major axes; (6) modifications and specializations of marginal teeth suggestive of as yet undocumented selective deposit-feeding strategies.

Some of the above patterns are similar to those documented in other groups of mollusks. The original studies of adaptive convergence were conducted by SOLEM (1971, 1972, 1973, 1974) on the radulae of land snails. His research elegantly established the utility of SEM in radular studies. Similar surveys are now available for cephalopods (SOLEM & RICHARDSON, 1975; SOLEM & ROPER, 1975) and nudibranchs (BERTSCH et al., 1973; BERTSCH & FERREIRA, 1974; FERREIRA & BERTSCH, 1975). Preliminary work on the rhipidoglossate radula has revealed a much more complex set of patterns. This is, in part, because the rhipidoglossate radula is mechanically and morphologically the most complex type of molluscan radula.

The typical rhipidoglossate radula is unique in possessing a large number of long slender marginal teeth, arrayed symmetrically on either side of the flat central portion of the ribbon. The marginal "books" of teeth are erected by outfolding of the ribbon and subsequently collapsed over the central part of the ribbon during the feeding stroke, as the ribbon passes over the tip of the odontophorial cartilage (ANKEL, 1938; EIGENBRODT, 1941; FRETTER, 1965; FRETTER & GRAHAM, 1962; NISBET, 1973; GRAHAM, 1973). The central part of the ribbon is specialized primarily for various kinds of food preparation and acts along an axis parallel to the length of the ribbon, while the marginals engage primarily in food gathering and act through a broad arc perpendicular to the length of the ribbon.

For both OM and SEM examination it is necessary to perform delicate manipulations on the radula (e. g., folding out or removing parts of the marginal books, separating longitudinal and transverse rows) in order to minimize overlap of teeth and observe individual tooth morphologies and interrelationships. Because SEM viewing requires dry specimens coated with a conductive metal while optical mounts are made in a fluid medium, different configurations inevitably result from the two basic kinds of preparation.

The most important single complementary difference between SEM and OM viewing of the rhipidoglossate radula is related to the transparent but differentially mineralized and tanned nature of the chitinous teeth and ribbon to which they are attached. Because SEM viewing is restricted to surface features, it is possible to eliminate the confusion of multiple overlapping that occurs in standard OM preparations. On the other hand, OM permits study of completely obscured teeth and parts of teeth that would never appear in SEM preparations as well as enabling observation of internal structure patterns related to readily visible differential mineralization and differential tanning revealed through staining.

LIGHT MICROSCOPY

The decrease in depth of field and resolving power that accompany increased magnification in optical systems are among the most serious problems in the traditional method of studying and illustrating radulae, although these are spectacularly minimized in the finest modern laboratory microscopes. Ease of varying illumination parameters, and, in particular, the use of color filters to bring out detail and contrast (Figure 8b), enhance the data gathering potential of such instruments and play a key role in black-and-white photomicrography. Problems of photomicrography related to adaptation of camera optics (SOLEM, 1970) are eliminated in trinocular systems utilizing built in photographic objectives and rapid means of establishing parafocality between visual image and film plane.

An additional, often cited, disadvantage of light microscopy lies in the fact that the radula must be mounted ("squashed," in the words of the most severe critics) between 2 pieces of glass and viewed from a single position. Limited angle of observation may be compensated by dissecting portions of the radula so that individual teeth are mounted in a variety of positions as well as by use of depression slides or coverslip supports so that the radula retains three-dimensionality within the medium.

As suggested in the introduction, the optical properties of radulae present both advantages and disadvantages. In the complex rhipidoglossate radula in particular, there is a great deal of overlapping of elements, making it time consuming and difficult to sort out the morphology and interrelationship of individual teeth. In careful optical preparations, however, it is possible to determine relationships by focusing at different levels within groups of teeth and individual teeth in a way that is not possible in SEM viewing, which is restricted to surface features. OM is of great importance in detecting structures that can be subsequently explored more effectively with SEM, but which do not appear in preliminary SEM preparations.

One of the most important advantages of OM study of the rhipidoglossate radula is the potential amount of preparation time available for mounting in a fluid medium. Using a relatively low-viscosity water-soluble medium (e. g., Turtox CMCP-10), one can spend an hour or more dissecting and arranging the radula before adding the cover slip. Even some of the more viscous media allow more preparation time than is available while drying a radula for SEM viewing. The more viscous the medium, the more likely that an arrangement will be preserved when the cover slip is added, although interesting and fortuitous configurations and structures may be revealed

by turning and rotating of elements that may result. Distortions resulting from shrinkage of the radular membrane during drying for SEM viewing may likewise be either detrimental or fortuitous, the important point being that the two methods of preparation inevitably provide different configurations offering different kinds of information.

Differences in the flexibility and hardness of different parts of a radula or of individual teeth must evolve in close relationship to the mechanical constraints on radular functioning and the feeding habits and substrates of individual species. Thus OM observations of mineralization and staining patterns are of importance in radular studies. Documentation of mineralization in radular teeth is primarily restricted to the more spectacular instances of polyplacophorans and docoglossate archaeogastropods in which salts of iron and silicon increase tooth hardness (Jones et al., 1935; Carefoot, 1965; Towe & Lowenstam, 1967; Runham et al., 1969), although various minerals may be added during the transformation of the soft first-formed radula into the toughened and hardened active portion of the radula (Gabe & Prenant, 1957, 1958). Differential tanning, or formation of protein crosslinkages within the primary chitin structure, is indicated by differential staining patterns. For example, tooth bases are often heavily stained, while the slender flexible shafts of long marginal teeth often do not stain at all.

There are three major graphic methods of presenting OM observations of radulae: the camera lucida drawing, the composite drawing or reconstruction, and the photomicrograph. Camera lucida drawings provide faithful outline reproductions of tooth proportions and are a simple rapid means of illustrating a single half-row of teeth for comparative taxonomic purposes. Such drawings do not incorporate variations within an individual or population, and, as line drawings of complex objects, they often contain a great deal of subjectivity or ambiguity, or both. They are most useful for showing cusps and seldom show details of tooth bases. Camera lucida drawings that attempt to show three-dimensional structure and overlapping are often difficult to interpret.

Composite line drawings and reconstructions also contain a strong subjective element, but if camera lucida is used to establish proportions, such drawings may be extremely useful as summaries of observations at a variety of magnifications, of different portions of a single radula, or of different radulae. Such reconstructions can incorporate data from electron scan images as well as light images.

Photomicrography is most useful for producing a "true" image of the radula. Light micrographs are not particularly informative, however, if there is too much loss of resolution and depth of field in the image. Few authors have published light micrographs of radulae, but see McLean, 1971, for a series of informative light micrographs of turrid radulae. Micrographs at lower magnifications are particularly efficient for documenting basic taxonomic characters (number of longitudinal rows of teeth, numbers and kinds of transverse elements, gross morphology, etc.). Micrographs are also an important means of documenting more detailed information and discoveries, and it should not matter that part of an image is blurred if the structure of specific interest is clearly resolved. Indications of differential strength and hardness in teeth resulting from tanning and mineralization are best recorded through light microscopy.

Alternative optical methods to standard absorption contrast may also be useful in radular study. Maes (oral communication, 1977) has experimented with low angle incident illumination to produce some remarkably three-dimensional photomicrographs of glycerine jelly mounts of rachiglossate radulae (see Orr, 1956; Maes, 1967). Phase contrast microscopy is extremely useful for producing in-focus contrast in unstained preparations (as opposed to the phase contrast that can be achieved in ordinary light microscopy by defocusing). Mills (1977) has published some excellent phase contrast photomicrographs of toxoglossate radulae. Nomarski optics may also prove useful for examining some aspects of surface contour and should be explored as Nomarski systems become more widely available in this country.

SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy has largely replaced light microscopy as the preferred tool for radula study during the past decade. Its most important advantages lie in broadening the potential scope of investigations, particularly at magnifications beyond the resolving power of light microscope optics.

In functional morphological studies, SEM has become an indispensible tool, not only in its ability to explore microtopography from various angles, but also in the inherent ability of the incident electron probe to penetrate and explore narrow fissures and deep cavities that cannot be exposed by light.

Research on functional morphology of the molluscan radula has focused on the obvious food-preparing and food-gathering operations of the teeth and their relationship to design. These aspects are ideally examined from different angles with SEM and require some knowledge of the radula in functioning position. Less attention has been paid to the more routinely mechanical economics of

occupying space. SOLEM (1971, 1974) and SOLEM & ROPER (1975) have recognized the need to fold teeth together efficiently to prevent interference with food passage and have identified several adaptations in land snails and cephalopods (grooving, curved fit surfaces) that function in tooth compaction. In the complex rhipidoglossate radula, occupation of space might be more profitably explored in terms of designs that simultaneously satisfy maximum coverage of space by erected marginal teeth (as a function of tooth lengths and degrees of arc covered) during sweeping activities and minimum occupation of space when collapsed. Interlocking and complex lock-and-key fits between tooth bases, shafts and cusps, viewed heretofore only as functioning in stress support, also appear to play an important role in the economics of space occupation, particularly in the complex rhipidoglossate radula of marine archaeogastropods. These relationships are most effectively investigated by contrasting SEM data from preparations with marginal teeth erected with OM data from preparations with teeth in overlapping and tightly folded position.

Although the great depth of field and high resolution of the electron scan image are impressive, aesthetically satisfying, and "state-of-the-art" in appearance, SEM images do not automatically contribute anything useful to comparative taxonomic research and can be detrimental and confusing if they have been produced from different viewing angles and cannot be compared. Mounting between 2 pieces of glass for optical microscopy produces one of the most standard methods for comparing radulae of closely related congeners or for studies of variation patterns within populations, species, or along the length of a single radula. If SEM is used in illustrating strictly taxonomic studies, comparability of images should be maintained by standardizing the viewing angle and mounting the radula flat.

Electron micrographs serve as the primary method of recording data during SEM viewing and are the usual means of illustrating published studies. Composite drawings based on a series of SEM images could be used to greater effect. Stereoscopic paired micrographs are invaluable in interpreting SEM imagery. Low magnification stereoscopic pairs of entire preparations are particularly helpful for planning efficient viewing strategies.

THE RADULA OF *Tricolia pullus*

The major features of the radula of the phasianellid *Tricolia pullus* are illustrated in Figures 1 to 4, 11, and 12.

I have chosen this radula for illustration for two reasons: (1) it has been known for nearly a century and has been figured by previous authors (TROSCHEL, 1878: plt. 18, fig. 10; PILSBRY, 1888: plt. 61, fig. 2; ROBERTSON, 1958: plt. 138, fig. 3) and (2) it contains several features that are difficult to interpret but potentially important in understanding basic functioning patterns of the rhipidoglossate radula.

Drawings of the *Tricolia pullus* radula based on light microscope examination (Figure 10) establish the basic pattern of dentition and overlap of an oval rachidian flanked on either side by 5 strongly cusped, progressively narrower laterals with expanded overlapping bases and series of numerous marginals. The inner marginals have

Explanation of Figures 1 to 9

Tricolia pullus (Linnaeus, 1758). USNM 179033

Figure *1*: Central portion of whole mount. OM   × 100
Figure *2*: Central and inner lateral teeth, illustrating transverse basal overlap system. SEM   × 570
Figure *3*: Detail of inner marginal cusp and rectangular "lateromarginal plate" with adjacent marginal teeth removed. OM   × 300
Figure *4*: Isolated inner marginal tooth, demonstrating that "lateromarginal plate" is the tooth base. Note that cusp was broken during manipulation. OM   × 300

Solariella nuda Dall, 1896. USNM 209300

Figure *5*: Detail of first lateral tooth from which cusp is broken, revealing basal interlock with central tooth (lower right) and basal interlock with second lateral tooth (upper left). SEM   × 760
Figure *6*: Detail of lateromarginal plates where marginal teeth have been removed. OM   × 160
Figure *7*: Detail of lateromarginal plates with first marginal teeth folded out, other marginals removed. OM   × 160
Figure *8*: Central portion of folded whole mount with a. normal illumination and b. red filter to enhance contrast. OM × 115
Figure *9*: Low angle side view showing a. erected marginal teeth; b. lateromarginal plate with thin membranous rudimentary cusp; c. cuspless lateromarginal plate; d. grooves and ridges on heavy outer lateral tooth; e. depression on rachidian accommodating cusp of following rachidian. SEM   × 225
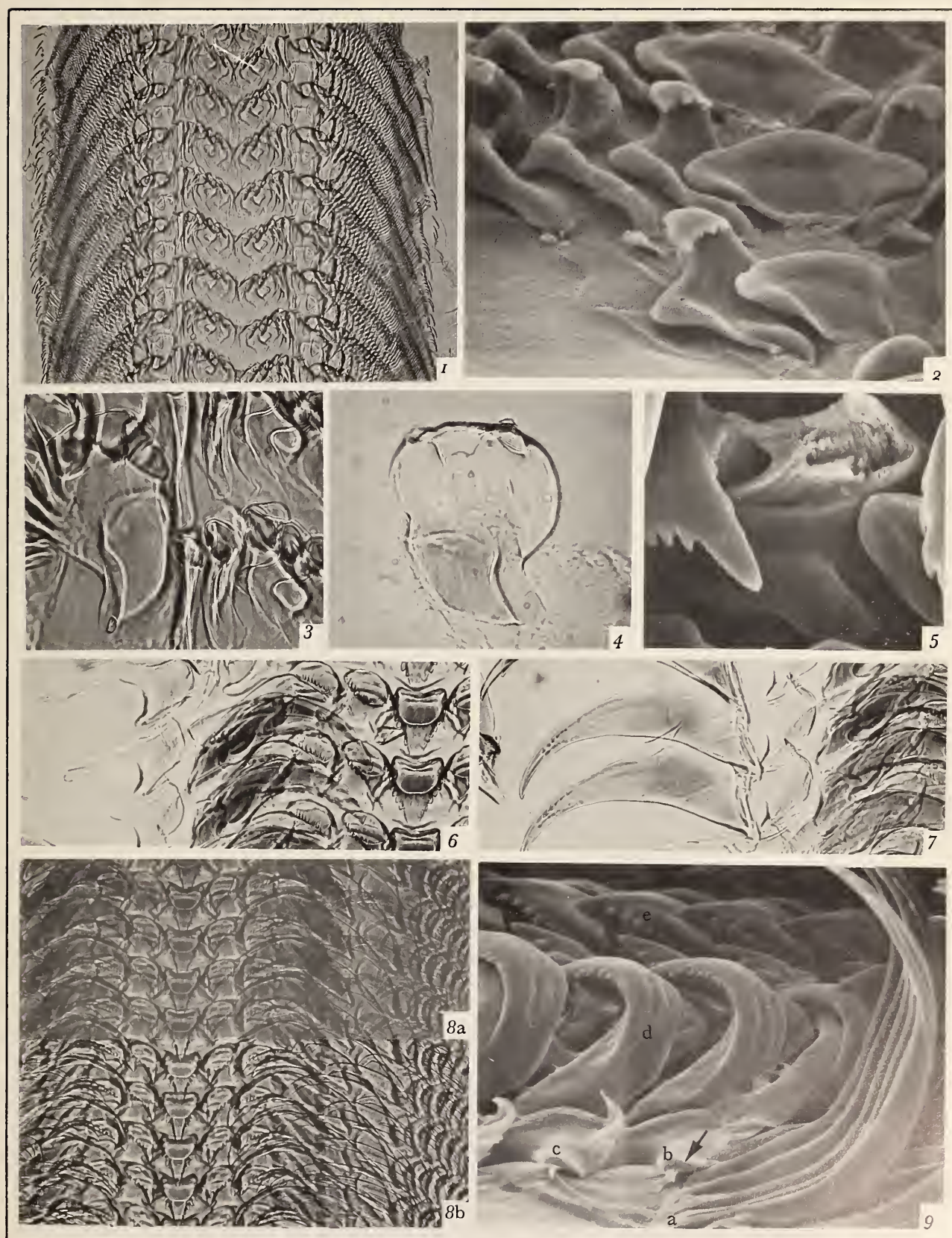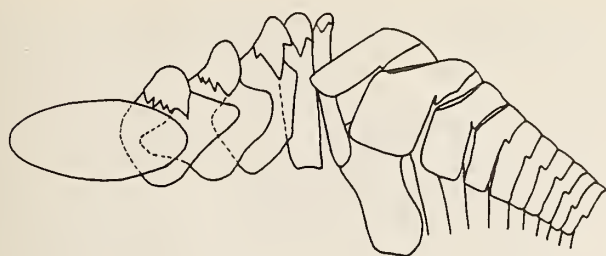
Figure 10

Line drawing of a part of a half-row of the radula of *Tricolia pullus*, based on light microscopy, after ROBERTSON (1955).
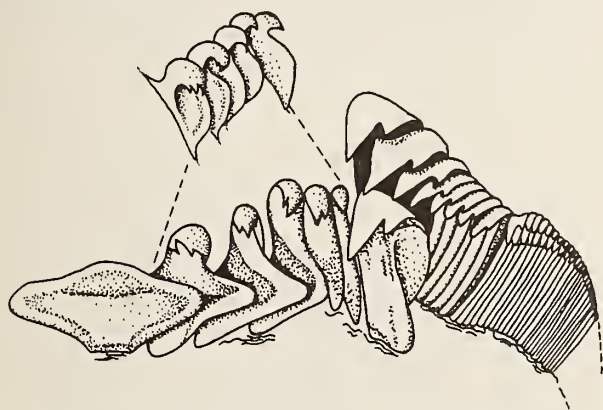

Figure 11

Reconstruction of part of a half-row of the radula of *Tricolia pullus*, from light and scanning electron microscope imagery. Inset of lateral teeth shows semi-profile view of cusps.

a strong primary cusp and a short secondary cusp along the outer margin. The outer marginals have a single, narrow, serrate cusp. For comparative taxonomic purposes, drawings of this kind provide excellent documentation from a standard vantage point of characteristic configurations or patterns (ROBERTSON, 1958: plt. 138, figs. 3 - 6). Although cusp details are depicted, the drawings are basically two-dimensional outlines that do not attempt interpretation of the thickness of teeth or parts of teeth, the manner in which teeth are attached to the radular ribbon, or the significance of overlapping elements.

A photomicrograph of the radula of *Tricolia pullus* (Figure 1) illustrates the kind of image upon which the line drawing of Figure 10 was based. The photomicrograph contains a great deal more complexity and additional kinds of information, the most obvious of which are

the patterns of multiple overlap of transverse rows and the variation in optical properties of different radular elements. Micrographs such as this and the comparable one for *Solariella nuda* (Figure 8) provide a rapid means of documenting sources of variation within longitudinal rows, including (1) random variation in actual morphology, as in the number of cusps, resulting from developmental differences, (2) random variation in apparent morphology resulting from viewing a tooth from a slightly different angle, and (3) systematic variation along the length of the ribbon resulting from tooth wear.

It is also evident that parts of the absorption contrast image are subject to more than one interpretation. A particularly interesting case of interpretational ambiguity arises from a prominent series of dark-staining rectangular structures between the outer lateral and inner marginal teeth in a whole mount of the *Tricolia* radula. ROBERTSON (1958) interpreted the structure as a cuspless tooth and proposed the name "lateromarginal plate" for it. MARCUS & MARCUS (1960) rejected Robertson's interpretation and suggested that the plate is the base of the innermost marginal tooth. Dissection of the radula to isolate individual teeth confirms the latter view, and photomicrographs of the first lateral tooth with its enlarged rectangular base appear in Figures 3 and 4.

Examination of large numbers of rhipidoglossate radulae, however, does reveal the presence of a cuspless structure between the lateral and marginal teeth in many archaeogastropods (Hickman, unpubl.), and the name lateromarginal plate is perfectly applicable. It is also interesting to note that there are a number of overlooked illustrations of and independent allusions to these cuspless structures in earlier literature (*e. g.*, SARS, 1878, *Margarites*; PILSBRY, 1889, *Trochus, Clanculus*; TROSCHEL & THIELE, 1891, *Moelleria, Puncturella*; SCHEPMAN, 1908, *Solariella*; TORR, 1914, *Emarginula, Lucapinella, Megatebennus*).

In many cases the plate is clearly a pivotal or articulatory structure, either supporting or holding the bases of the marginal books of teeth as they are erected and collapsed during feeding. In several trochid genera (*e. g.*, *Margarites, Calliotropis, Bathybembix*) rudimentary cusps are associated with the plate, suggesting its independent derivation through progressive enlargement of the base and reduction and loss of the cusp (HICKMAN, in preparation).

SEM examination adds significantly to our understanding of the *Tricolia* radula, particularly in interpretation of overlapping structures. Figure 2 reveals a complex and relatively heavy basal stress support system whereby the stress applied to any one tooth may be spread outward to other teeth within the same transverse row. In addition to the basal overlap features, Figure 2 also reveals inter-

lock features higher on the teeth that are more likely to function in compaction of the radula when it is not in use. Note the potential fit of the thickened convex ridge on the shaft of the third lateral into the corresponding pocket beneath the cusp of the second lateral in Figure 2.

A reconstruction of the *Tricolia* radula (Figure 11), based on a series of 7 SEM images from a variety of angles and magnifications, including 2 stereo pairs, also shows a complex system of interlocking of the inner marginal cusps in which the secondary outer cusp fits into a groove on the flattened top of the neighboring primary cusp.

THE RADULA OF *Solariella nuda*

The radula of *Solariella nuda* has not been figured previously. Radulae have been illustrated by line drawings for at least 15 species of *Solariella*, however (SARS, 1878; TROSCHEL, 1878; MARTENS & THIELE, 1903; SCHEPMAN, 1908; ODHNER, 1912; POWELL, 1951; GALKIN, 1955; McLEAN, 1964), and some of the major features of the solarielline radula, as heretofore understood, are illustrated in a line drawing of *Solariella delicata* Dall (Figure 12).

Recurrent characteristic features of the line drawings in the literature and features cited in accompanying texts, all based on optical examination, include (1) the shortness of the radula, which is sometimes less than twice as long as it is wide; (2) the small number of marginal teeth (approximately 10 per half-row); (3) the prominent dip at the center of each transverse row; (4) the prominent, coarsely serrated cusp of the rachidian; and (5) the apparent restriction of serrations to the outer margins of the first and second lateral teeth.

Other features are ambiguous or variably interpreted from one drawing to another, particularly with respect to basal overlapping and the shapes of the third, fourth, and fifth lateral teeth, if these are recognized as being present.

The reason for the ambiguity lies in the fact that the marginal teeth in the folded solarielline radula multiply overlap and obscure all but the rachidian and inner 2 lateral teeth in an unmanipulated preparation. This is illustrated by a photomicrograph of the folded radula of *Solariella nuda* (Figure 8).

When the marginal books of teeth are folded out or carefully removed, a broad-based third lateral tooth with a serrate, long, medially-directed cusp is revealed, as well as a narrow but heavily based and extraordinarily long, cusped fourth lateral tooth. In addition there is a longitudinal series of rectangular lateromarginal plates
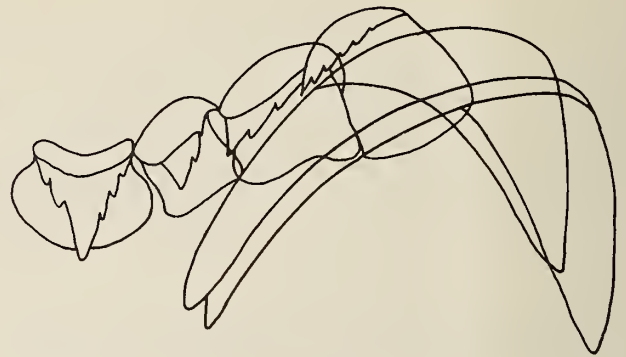

Figure 12

Line drawing of a half-row of teeth from the radula of *Solariella delicata*, redrawn from GALKIN (1955) and retaining ambiguity of line from his drawing.
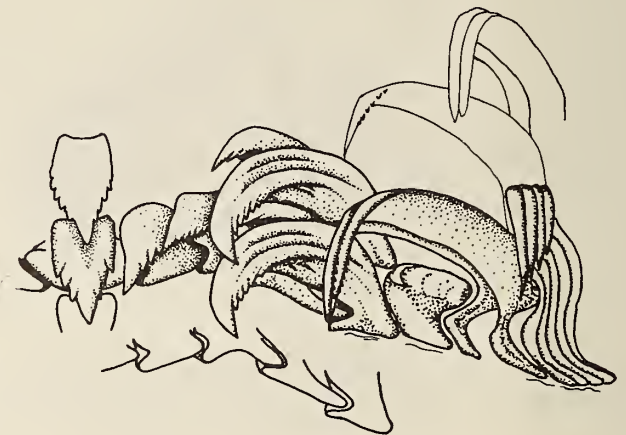

Figure 13

Reconstruction of the basic solarielline dentition pattern as exemplified by *Solariella nuda,* from light and scanning electron microscope imagery. A diagrammatic representation of the basal interlock system appears beneath the main part of the reconstruction.

(Figures 6, 7) against which the marginal books articulate. I have found similar series of lateromarginal plates in 7 other solarielline species, although SCHEPMAN (1908: plt. 9, figs. 8, 9) is the only author to have recorded them.

A SEM preparation in which the marginal teeth have been removed for viewing from the side (Figure 9) presents an alternative view of the lateromarginal plates of *Solariella nuda.* Note that, although the posterior 2 plates have a small hooked projection on their inner margins,